Spindle pole body components are reorganized during fission yeast meiosis

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ABSTRACT **During meiosis, the centrosome/spindle pole body (SPB) must be regulated in a manner distinct from that of mitosis to achieve a specialized cell division that will produce gametes. In this paper, we demonstrate that several SPB components are localized to SPBs in a meiosis-specific manner in the fission yeast *Schizosaccharomyces pombe*. SPB components, such as Cut12, Pcp1, and Spo15, which stay on the SPB during the mitotic cell cycle, disassociate from the SPB during meiotic prophase and then return to the SPB immediately before the onset of meiosis I. Interestingly, the polo kinase Plo1, which normally localizes to the SPB during mitosis, is excluded from them in meiotic prophase, when meiosis-specific, horse-tail nuclear movement occurs. We found that exclusion of Plo1 during this period was essential to properly remodel SPBs, because artificial targeting of Plo1 to SPBs resulted in an overduplication of SPBs. We also found that the centrin Cdc31 was required for meiotic SPB remodeling. Thus Plo1 and a centrin play central roles in the meiotic SPB remodeling, which is essential for generating the proper number of meiotic SPBs and, thereby provide unique characteristics to meiotic divisions.**

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INTRODUCTION

The spindle pole body (SPB) is a structure equivalent to the centrosome in fungi. It functions as a microtubule-organizing center (MTOC) at which spindle microtubules begin assembling during mitosis (Snyder, 1994; Adams and Kilmartin, 2000; Wiese and Zheng, 2006). Another recently discovered feature of centrosome/SPB is that it is an organelle involved in many cellular processes, such as cell cycle progression, DNA damage checkpoint, and asymmetric cell division (Fletcher and Muschel, 2006; Bornens, 2012).

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Abbreviations used: γ-TuC, γ-tubulin complex; CDK, cyclin-dependent kinase; CFP, cyan fluorescent protein; DAPI, 4,6-diamidino-2-phenylindole; DSR, determinant of selective removal; EMM, minimal medium; GCP, γ-tubulin complex proteins; GFP, green fluorescent protein; MAP, mitogen-activated protein; MF, mating factor; MI, meiosis I; MII, meiosis II; MTOC, microtubule-organizing center; Plo1-Sid4C, Sid4C-fused Plo1; Sid4C, C-terminal portion of Sid4; SIN, septation initiation network; SPA, sporulation agar; SPB, spindle pole body; YEA, yeast extract medium supplemented with adenine.

In the fission yeast *Schizosaccharomyces pombe*, SPBs are located close to the cytoplasmic side of the nuclear envelope during interphase (Ding *et al*., 1997), and duplicated SPBs are embedded within the nuclear envelope upon the cell's entry into the mitotic cycle. SPBs consist of many types of proteins and thus display a wide variety of functions. In the budding yeast *Saccharomyces cerevisiae*, ultrastructural observations revealed that the main body of an SPB consists of three electron-dense layers: a cytoplasmic outer plaque, a central plaque in the transmembrane zone, and a nuclear inner plaque (Adams and Kilmartin, 2000). Each SPB component is located within one of these layers and plays a specific role at the cytoplasmic or nuclear side or as a structural constituent. The main body of an SPB is accompanied by a half-bridge structure, which is an extension of the SPB substructure and contains Sfi1 and the yeast centrin orthologue Cdc31 (Kilmartin, 2003). The half-bridge structure is required for SPB duplication, as Sfi1 and Cdc31 mutants exhibit defects in SPB duplication in yeasts (Kilmartin, 2003; Paoletti *et al*., 2003; Araki *et al*., 2010).

Because the SPB is an MTOC, it contains a γ-tubulin complex (γ-TuC), which is a microtubule nucleator composed of γ-tubulin and γ-tubulin–associated proteins designated as GCPs (γ-tubulin complex proteins). These proteins are conserved among eukaryotes (Kollman *et al*., 2011). γ-TuCs are recruited and tethered to MTOCs. In fission yeast, a coiled-coil protein, Pcp1 (Spc110p in budding

yeast), is essential for recruiting γ-TuC to SPBs (Flory *et al.*, 2002; Fong *et al.*, 2010). It is also essential for nucleating spindle microtubules, although Pcp1 is not required for nucleation of cytoplasmic microtubules (Kilmartin and Goh, 1996; Flory *et al.*, 2002; Fong *et al.*, 2010). Thus the function of Pcp1 as an MTOC component is exerted at the nuclear surface of SPBs after SPBs are embedded in the nuclear envelope. Although Pcp1 function is restricted to the mitotic phase of the cell cycle only, Pcp1 is constantly localized to SPBs (Flory *et al.*, 2002; Fong *et al.*, 2010).

There are several components that have M-phase–specific functions and localize to SPBs only during mitosis, for example, Cut11 (West *et al.*, 1998) and Msd1 (Toya *et al.*, 2007). Other transient SPB components are mostly related to cytokinesis at mitotic exit. Two daughter cells are separated by a septum created at the cell center. The septum is induced by a cascade of kinases called the septation initiation network (SIN), which corresponds to the mitotic exit network pathway in *S. cerevisiae* (Bardin and Amon, 2001; McCollum and Gould, 2001; Krapp and Simanis, 2008). Many SIN components, such as Spg1, Byr4/Cdc16, Cdc11, the NDR kinase Sid2, and Sid4, also localize to SPBs during interphase (Sohrmann *et al.*, 1998; Cerutti and Simanis, 1999; Sparks *et al.*, 1999; Krapp *et al.*, 2001; Tomlin *et al.*, 2002), whereas Sid1 and Cdc7 localize to SPBs only during mitosis (Sohrmann *et al.*, 1998; Guertin *et al.*, 2000).

The other NDR (nuclear DBF2-related) kinase Orb6 is a member of the MOR (Morphogenesis Orb6 Network) signaling cascade (Verde *et al.*, 1998; Tamaskovic *et al.*, 2003; Kanai *et al.*, 2005). The MOR signaling pathway is essential for polarized growth in interphase. The MOR pathway is composed of proteins that include the orthologue of human MO25 protein, Pmo25 (Kanai *et al.*, 2005; Mendoza *et al.*, 2005); the GC kinase Nak1 (Kanai *et al.*, 2005; Leonhard and Nurse, 2005); the homologue of *Drosophila* Furry, Mor2 (Hirata *et al.*, 2002); and in addition to Orb6, which is the most downstream kinase on the pathway known to date. Pmo25 and Nak1 proteins localize to both of the two SPBs in early mitosis and only to the new SPB in anaphase (Kanai *et al.*, 2005; Mendoza *et al.*, 2005). Thus the SPB is essential as a platform for a number of factors regulating cytokinesis and polarized growth.

SPBs also play essential roles during sexual differentiation. Removing a source of nitrogen triggers sexual differentiation in *S. pombe*, namely conjugation of cells (mating) and meiotic processes (Yamamoto *et al.*, 1997). This includes premeiotic DNA replication, meiotic prophase for meiotic recombination and two consecutive nuclear divisions, meiosis I (MI) and meiosis II (MII), followed by sporulation (see schematic in Supplemental Figure S1). After the mating of the two cells with different mating types (h^+ and h^-), the zygote undergoes karyogamy, in which two nuclei derived from the parental cells fuse into one (Yamamoto *et al.*, 1997). Karyogamy occurs via fusion of the two SPBs, which is guided by an astral array of cytoplasmic microtubules connected to SPBs (Rose, 1991). After nuclear fusion, the microtubule array tethered to the SPB causes the nucleus to move back and forth. This SPB-driven nuclear oscillation, specific to meiotic prophase, is called horse-tail nuclear movement and is essential for promoting meiotic recombination of homologous chromosomes (Chikashige *et al.*, 1994; Ding *et al.*, 1998; Yamamoto *et al.*, 1999). An SPB protein, Hrs1 (also called Mcp6), localizes to the SPB only during this stage and anchors the cytoplasmic array of microtubules to it, which is necessary for the oscillatory movement (Saito *et al.*, 2005; Tanaka *et al.*, 2005).

Although female meiosis is acentrosomal in a number of higher eukaryotes (González *et al.*, 1998; Compton, 2000), spermatogenesis and fungal meiosis generally require centrosomes and SPBs, respectively (Schatten, 1994; Jaspersen and Winey, 2004). To achieve meiosis-specific differentiation, it is obvious that SPBs and microtubules need to be regulated in unique ways. Despite their biological uniqueness and importance, little is known regarding how SPBs are regulated during meiosis. We postulated that meiosis-specific regulation of SPBs could contribute to generating meiosis-specific cell cycle progression. During mitosis, SPBs make an obvious contribution to cell cycle progression, from G2 to M phase. In eukaryotes, entry into mitosis is regulated by the cyclin-dependent kinase complex Cdk1-cyclin B and the polo kinase (Cdc2-Cdc13 and Plo1 in *S. pombe*; Nurse, 1990; MacIver *et al.*, 2003; Takaki *et al.*, 2008). Both Cdc2-Cdc13 and Plo1 localize to SPBs upon entry into the mitotic cycle (Bähler *et al.*, 1998a; Decottignies *et al.*, 2001); this subsequently promotes numerous mitotic events. The first event during entry into the mitotic cycle is localization of Cdc2-Cdc13 at SPBs, whose activity is required to activate Plo1 (Tanaka *et al.*, 2001). It was recently reported that Pcp1 promoted localization of Plo1 to mitotic SPBs. Thus Pcp1 recruits not only γ-TuC but also Plo1 to mitotic SPBs, thereby exerting a novel function for advancing entry into mitosis (Fong *et al.*, 2010). Plo1 localized at SPBs is further activated by an SPB component, Cut12 (MacIver *et al.*, 2003), which binds to Plo1. This in turn activates Cdc2-Cdc13 via a positive-feedback mechanism.

Plo1 activity is also essential for promoting fission yeast meiosis. Interestingly, it was recently reported that Plo1 kinase localized to kinetochores during horse-tail nuclear movement during meiotic prophase (Krapp *et al.*, 2010). This indicates that Plo1 must be relocated from SPBs and activated at kinetochores in order to play some as yet unidentified function. We assumed that the meiosis-specific localization pattern of Plo1 might be a clue to how SPBs are regulated during meiosis and how Plo1 affects meiotic cell cycle progression.

Therefore, in this study, we reexamined the localization of Plo1 in order to determine the reason for Plo1 exclusion from the SPB until the late stage of meiotic prophase. We found that SPBs are remodeled in a meiosis-specific manner. Several SPB components dissociated from SPBs during prophase and returned to SPBs immediately before the onset of MI. Furthermore, we found that Plo1 plays an essential role in the remodeling of SPBs upon MI entry, and this appears to account for why Plo1 needs to be excluded from the SPB.

RESULTS

Plo1 is excluded from SPBs during meiotic prophase

To investigate how SPBs are controlled during meiosis, we first monitored the behavior of Plo1 during meiotic progression. An endogenous copy of *plo1* was tagged with two tandem copies of green fluorescent protein (GFP), and Plo1-2GFP expression was observed along with an SPB marker, Sfi1–cyan fluorescent protein (Sfi1-CFP). During vegetative growth, localization of Plo1 was observed specifically in the M phase and was spatially restricted to SPBs and the nucleoplasm in order to regulate multiple aspects of mitotic events from early to late mitosis. As reported previously (Krapp *et al.*, 2010), Plo1-2GFP was specifically localized at kinetochores in the nucleoplasm, but not at SPBs during meiotic prophase (Figure 1A). Association of Plo1 to SPBs was observed in a later stage, namely at the onset of MI, as observed in mitosis (Figure 1A, 18–30 min). This indicates that Plo1 may play a role at kinetochores during meiotic prophase but is clearly excluded from SPBs during meiosis-specific prophase, which is when the horse-tail nuclear movement occurs. Even when Plo1-GFP was overexpressed during meiotic prophase, it did not preferentially localize to SPBs (Figure 1B). Thus the possibility that the expression of Plo1 was low during prophase was ruled out, suggesting strongly that the affinity of Plo1 for SPBs was reduced at this stage.

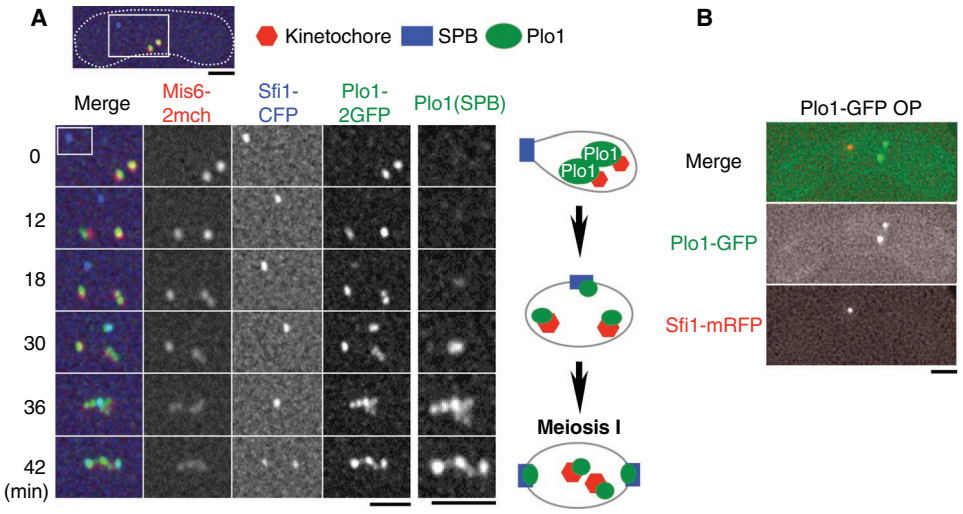

FIGURE 1: Plo1 is excluded from SPB during meiotic prophase. (A) Localization of Plo1-2GFP (green), Mis6-2mCherry (a kinetochore protein; red), and Sfi1-CFP (an SPB half-bridge protein; blue) during meiotic prophase. Top, a zygotic cell in meiotic prophase expressing these fluorescent protein-tagged proteins. The dotted line traces the cell shape. The inset, including the nucleus, was magnified, and the time-lapse images from that time point are shown below. Filming started at 0 min. The inset at 0 min corresponding to the SPB region is further magnified and shown at the right (Plo1(SPB)). Plo1 localized to kinetochores and was excluded from an SPB during prophase (0 min) and then started to localize to an SPB in a later stage (at MI onset, 18–30 min). This behavior was seen in all the observed cells (n = 6). Schematic drawings for the location of Plo1, kinetochores, and SPBs are shown at the far right. (B) Plo1-GFP was overproduced during meiotic prophase in Sfi1-mRFP cells (62% of cells, n = 45). Scale bars: 2 μm.

SPB components are differentially retained at SPBs during meiosis

We aimed to determine how Plo1 is excluded from SPBs during meiotic prophase, and what the biological significance of this control is. For this purpose, we expanded our multicolor fluorescence live-cell imaging to include two other SPB components, Cut12 and Pcp1. Cut12 activates Plo1 kinase (MacIver *et al.*, 2003), and Pcp1 shares homology with *Drosophila* centrosomin and is required for localization of γ-tubulin to SPBs and the recruitment of Plo1 during mitosis (Heuer *et al.*, 1995; Flory *et al.*, 2002; Fong *et al.*, 2010). Both Cut12 and Pcp1 are essential in meiosis (see Figure S6A) as well as in mitosis. Unexpectedly, Pcp1-GFP and Cut12-GFP did not localize to SPBs during meiotic prophase (Figure 2A). This was in sharp contrast to mitosis, in which these components are constantly localized at SPBs. These SPB components disappeared when the cells were committed to sexual differentiation (see Figure 3A later in the paper) and remained absent from SPBs for ~2 h throughout meiotic prophase while the cells underwent horse-tail nuclear movement. Then, immediately prior to MI, these factors, including Plo1, Pcp1, and Cut12, reappeared at SPBs (Figure 2, B and C; data not shown for Cut12). Based on localization kinetics during meiosis, SPB components that remain during mitosis were categorized into three groups (summarized in Table 1). In addition to Pcp1 and Cut12, the coiled-coil protein Spo15 (Ikemoto *et al.*, 2000) disappeared in meiotic prophase (these proteins are categorized as type I in Table 1; Figure S2A). Some components, such as Sid4 and Cdc11, showed reduced localization to SPBs during meiotic prophase (type II in Table 1; Supplemental Figure S2, B and C). Half-bridge proteins Sfi1 and Cdc31 (Kilmartin, 2003; Paoletti *et al.*, 2003) remained at SPBs (type III, Table 1). Type III also included Sad1, whose budding yeast orthologue (Mps3) is known to localize in close proximity to Cdc31 and Sfi1 (Jaspersen *et al.*, 2006), and Ppc89, which is thought to be a core structural component of SPBs (Rosenberg *et al.*, 2006; Figure S2D). Unlike these SPB components, Hrs1 is known to be expressed specifically during meiotic prophase (Figure 2A; Saito *et al.*, 2005; Tanaka *et al.*, 2005).

Type	Component behavior
I	Disappeared from SPBs: Cut12, Pcp1, Spo15
II	Showed reduced localization to SPBs: Sid4, Cdc11
III	Constantly observed at SPBs: Cdc31, Sfi1, Sad1, Ppc89

The known SPB components that constantly localize to SPBs during mitotic cell cycle are classified into three categories, based on behavior during meiotic prophase.

TABLE 1: Classification of SPB components based on their behavior during meiotic prophase.

A previous study has shown that Sid4 and Cdc11, as well as other mitotically constant SIN factors, localize to SPBs during meiotic prophase (Krapp *et al.*, 2006). Spo15 is reported to localize to SPBs during meiotic prophase, as seen in immunofluorescence images of fixed cells (Ikemoto *et al.*, 2000). Our live-cell recording, however, revealed that these components also alter their localization during meiotic prophase. These results demonstrate that the mitotically constant SPB components, including SIN members, are dynamically reorganized through meiotic progression.

The relocalization of Pcp1 (and other factors) was concomitant with the cessation of nuclear oscillation and the gradual disappearance of Hrs1. This indicates that the SPB components are rearranged in order to prepare for upcoming MI. Moreover, Pcp1 reaccumulated to SPBs simultaneous with Plo1 kinase, implying a possible link between SPB reorganization and association of Plo1 to SPBs (Figure 2C).

Mitogen-activated protein kinase signaling is responsible for the disappearance of SPB components

We next focused on how Cut12 and Pcp1 are regulated during meiosis by analyzing at what stage they dispersed from SPBs. Sexual

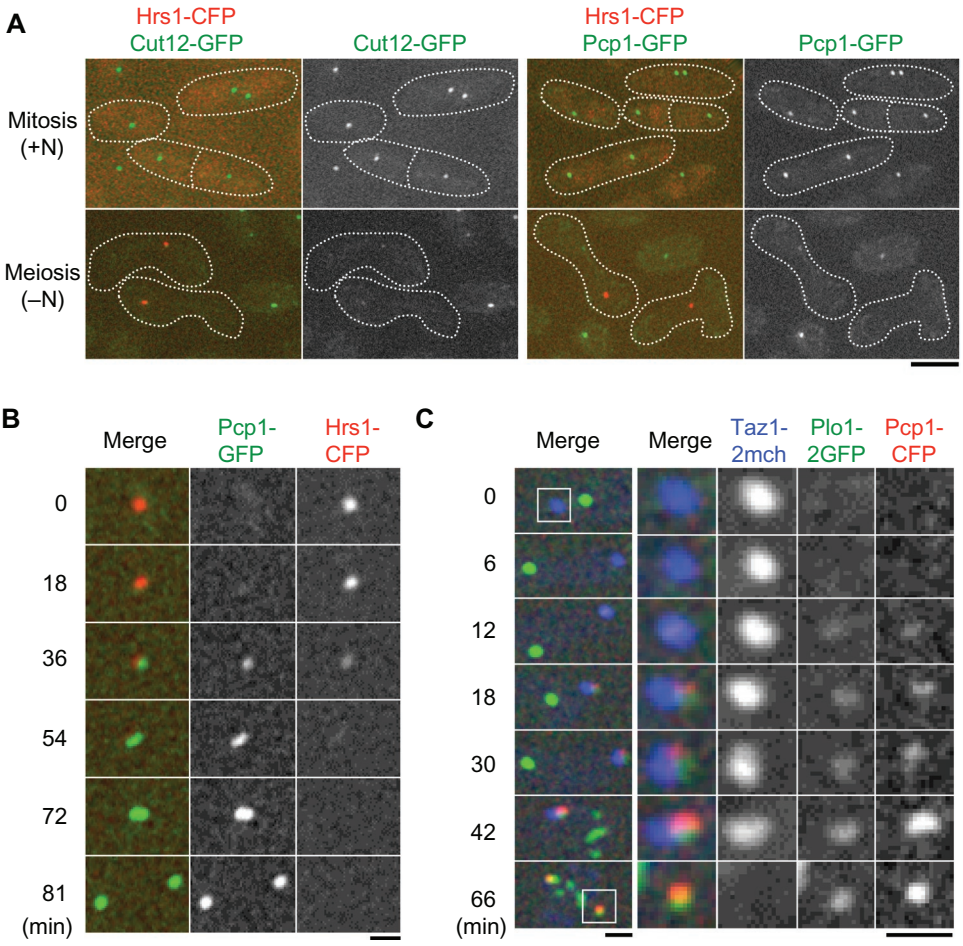

FIGURE 2: SPB is reorganized during meiosis. (A) The SPB components Cut12 and Pcp1 did not localize to SPBs during meiotic prophase. Cut12-GFP Hrs1-CFP (left) and Pcp1-GFP Hrs1-CFP (right) cells were cultured in media either with (+N) or without (–N) a nitrogen source to allow observation of these proteins during mitotic and meiotic cell cycles, respectively. Dotted lines trace the cell shapes and septa. Percentages of cells that lost the localization at SPBs are 96.4% for Cut12-GFP (n = 28) and 96.6% for Pcp1-GFP (n = 29). (B) Time-lapse images for Pcp1-GFP and Hrs1-CFP at SPBs in the transition from meiotic prophase to MI. Note that the Pcp1-GFP signals appeared at the SPB at 36 min immediately before entry into MI, after which the Pcp1-GFP dot was duplicated (54 min) and separated (after 72 min). This localization pattern was seen in all cells we observed (n = 15). (C) Simultaneous SPB localization of Plo1-2GFP and Pcp1-CFP to the onset of MI was observed (n = 4/4). The inset corresponding to the SPB region is shown magnified at the right with individual color channels. The signals for the telomere protein Taz1-2 mCherry clustered at the SPB until 42 min, but then dispersed from the SPB after entering MI (66 min). Scale bars: 5 μm (A) or 1 μm (B and C).

differentiation of *S. pombe* cells is induced by removing a nitrogen source from their nutritional medium. Nitrogen starvation induces conjugation of haploid cells, which is followed by meiotic prophase and meiotic divisions. Conjugation is initiated by a mutual give-and-take of mating factors (MFs; e.g., mating pheromones), which triggers the activation of the Spk1 mitogen-activated protein (MAP) kinase signaling cascade (Yamamoto *et al.*, 1997, 2004). Because the dispersion of the SPB components was observed during the early stage of sexual differentiation, we tested the involvement of the MF-responsive MAP kinase pathway by using heterothallic haploid cells in which this pathway was not activated under normal culture conditions.

We compared the intensities of Cut12-GFP signals in heterothallic h^- cells in the presence and absence of a nitrogen source. The Cut12-GFP signals remained at SPBs, indicating that removal of the nitrogen source itself does not trigger Cut12-GFP dispersion (Figure 3A, a and b). We then expressed the MF of h^+ cells ectopically in h^- cells to artificially induce Spk1 MAPK signaling. Cut12-GFP dispersed in these cells upon nitrogen starvation (Figure 3A, c and d). This dispersion was not observed when the signaling was blocked by Spk1 knockout (Figure 3A, e and f), confirming that MF-responsive MAP kinase signaling was essential for triggering the dispersion of the SPB component Cut12. Essentially the same conclusion was drawn with Pcp1 and Spo15, and also with Sid4, albeit dispersion was partial in the last case (Figure S3).

Cdk1 activity is required for reaccumulation of the SPB components

While dispersion of the SPB components occurs upon commitment to sexual differentiation, reaccumulation to SPBs occurs shortly before the onset of MI. Therefore it is possible that the activities of mitotic kinases may trigger reaccumulation of the SPB components. We examined whether the cyclin-dependent kinase Cdc2/Cdk1 was involved in this process. We used *cdc2-as* (analogue-sensitive) mutant, as it is convenient for live-cell imaging, the reason being that the activity of Cdc2 in this mutant can be inhibited immediately after ATP-analogue molecules are added to the medium (Bishop *et al.*,

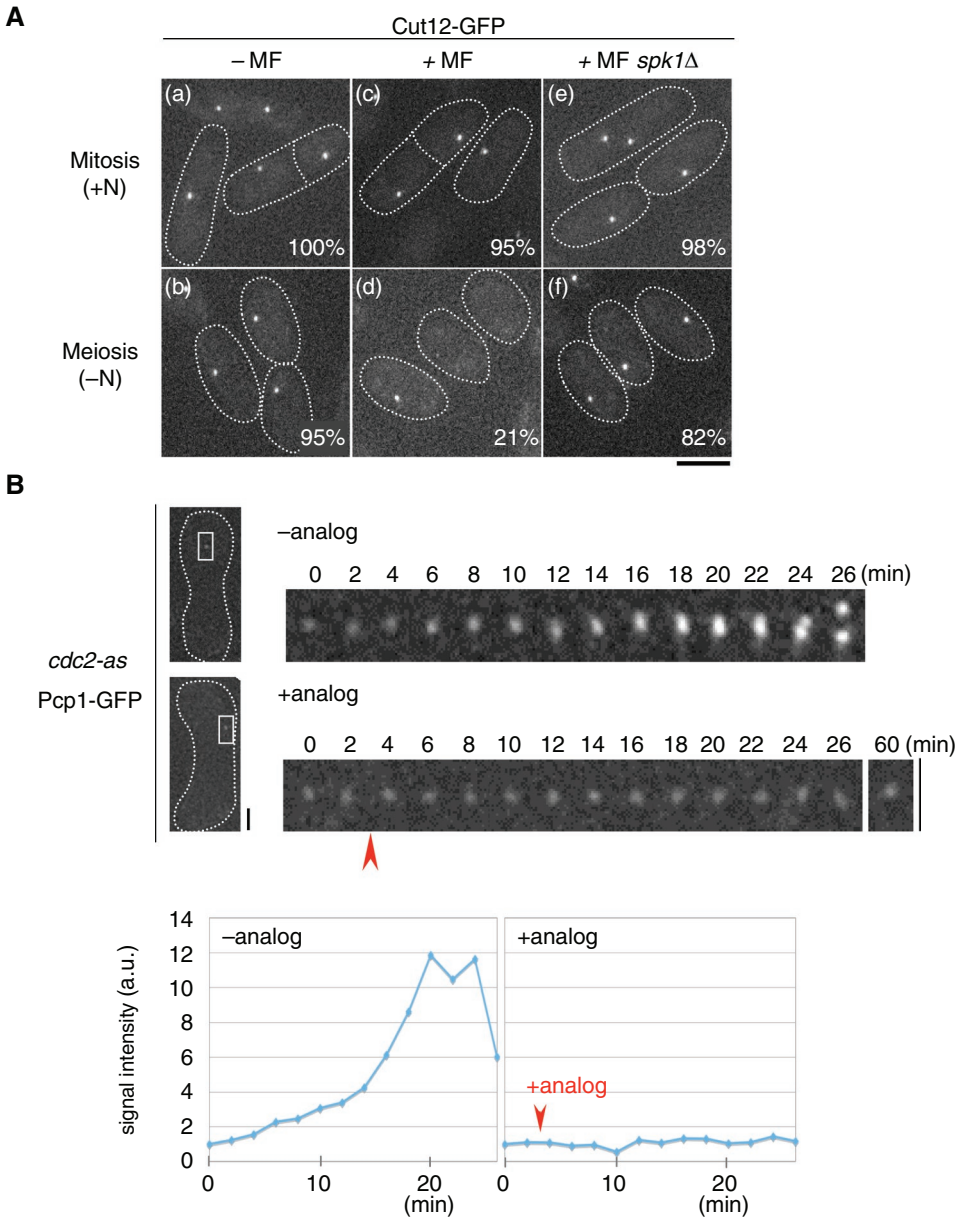

FIGURE 3: The MAP kinase Spk1 and the cyclin-dependent kinase Cdc2 are required for the dispersion and reaccumulation of SPB components, respectively. (A) Haploid cells expressing Cut12-GFP were inoculated in media either with (+N; a, c, and e) or without (–N; b, d, and f) a nitrogen source to allow observation of cells during mitotic and meiotic cell cycles, respectively. (a and b) Wild-type h^- cells, which do not express the MF of h^+ cells, express the other mating type (–MF). (c–f) h^- cells ectopically expressing the MF of h^+ cells (+MF) in the *spk1*$^+$ (c and d) and *spk1Δ* (e and f) background. Activation of the MF-responsive MAP kinase Spk1 is sufficient to disperse Cut12-GFP. Percentages of cells that showed SPB localization of Cut12-GFP are also shown ($n \geq 81$). Scale bars: 5 μm. (B) Meiotic prophase/MI transition of the *cdc2-as* (analogue-sensitive) mutant expressing Pcp1-GFP. When the Pcp1-GFP dot started to relocalize to SPB at the onset of MI, the ATP analogue 1NM-PP1 was added (arrowhead) and time-lapse images were acquired (+analogue, $n = 9$). A mock-treated cell is also shown (–analogue, $n = 6$). The signal intensity of Pcp1-GFP of representative cells was quantified and is shown below. Scale bars: 2 μm.

2000; Dischinger *et al.*, 2008; Sato *et al.*, 2009b). Because the original *cdc2-as* mutant is known to be meiotically deficient (Dischinger *et al.*, 2008), we created a new *cdc2-as* mutant that exhibited no meiotic defects (unpublished data) and used this in a drug assay.

In mock-treated cells, Pcp1 accumulated gradually upon entry into MI (Figure 3B, –analogue), while the signals stopped increasing upon the addition of an inhibitor (Figure 3B, +analogue). Moreover, overexpression of Cdc25 phosphatase, the activator of Cdc2, caused precocious localization of Cut12 at SPBs (Figure S4). Thus we concluded that Cdc2 activity was essential for reorganization of the SPB components.

SPB half-bridge is required for SPB remodeling at the onset of MI

In addition to the requirement for Cdc2 kinase activity, SPB reorganization is likely to require a structural anchorage that physically

aids reassociation of the "lost" components. Because a number of the SPB components are removed from SPBs during meiotic prophase, such a structural anchorage may tend to persist as opposed to a tendency to dissociate. After a visual screening of the strain collection of the SPB components labeled with GFP (Table 1), we focused particularly on the half-bridge structure of SPB, because Cdc31 and Sfi1, the two half-bridge components identified in fission yeast (Kilmartin, 2003; Paoletti *et al*., 2003), persisted at SPBs during meiotic prophase (type III in Table 1; Figures 1A and 4A). The half-bridge structure, which is juxtaposed to the main body of SPB, is known to regulate SPB duplication (Jaspersen and Winey, 2004), although the precise duplication mechanisms remain unclear.

To investigate the possible requirement of the half-bridge structure for SPB reorganization, we isolated temperature-sensitive mutants of *cdc31*. A *cdc31.ts10* mutant had meiotic defects at the restrictive temperature of 32°C, exhibiting abnormal chromosome segregation (Figures 4B and S5A). The effects of this mutation on SPB reorganization at the onset of MI were monitored by live-cell imaging. In wild-type cells, the Hrs1-CFP signals at SPBs diminished ~20 min after the cessation of nuclear oscillation at the end of meiotic prophase (Figure 4C, duration A). Reappearance of the Pcp1-GFP signals at SPBs occurred almost simultaneously with cessation of nuclear oscillation (Figures 4C, duration B, and S5B). Importantly, in *cdc31.ts10* cells, the Hrs1-CFP signals remained for a longer time (30–60 min), even after cessation of nuclear oscillation (Figures 4C, duration A, and S5B). Moreover, most *cdc31.ts10* cells exhibited an extended delay in relocalization of Pcp1-GFP to SPBs (Figures 4C, duration B, and S5B). Similarly, there was a significant delay in localization of Plo1-2GFP to SPBs in the *cdc31.ts10* mutant (Figure 4D). Thus the half-bridge structure of SPBs is important for SPB reorganization at the onset of MI, presumably as a structural anchorage for regathering the SPB components and for removing prophase-specific factors.

Ectopic localization of Plo1 to SPBs can recruit Pcp1 and Cut12

Plo1 requires Pcp1 and Cut12 for its function in mitotic cells (MacIver *et al*., 2003; Fong *et al*., 2010). They are also required for meiosis, as shown in Figure S6A. It has been reported that Cut12 interacts with Plo1 during mitosis and that a gain-of-function mutation of Cut12 causes precocious activation of Plo1 (MacIver *et al*., 2003). The *pcp1-18* mutant showed reduced localization of Plo1 to SPBs, suggesting the requirement of Pcp1 for recruiting Plo1 to SPBs (Fong *et al*., 2010). From this analogy, we hypothesized that the absence of Cut12 and Pcp1 during meiotic prophase might be a reason for the exclusion of Plo1 from SPBs. To test this possibility, we artificially tethered Cut12 or Pcp1 to SPBs during meiotic prophase by making a fusion of these factors with the prophase-specific component Hrs1. The Hrs1-Cut12 fusion protein successfully localized to SPBs during prophase (Figure 5A). Interestingly, Pcp1 was precociously recruited to SPBs in accordance with Hrs1-Cut12, indicating that Cut12 could recruit Pcp1 to SPBs (Figure 5A). Similarly, the fusion of Pcp1 to Hrs1 (Hrs1-Pcp1) recruited Cut12 to SPBs, indicating that Cut12 and Pcp1 can recruit each other reciprocally to SPBs during meiotic prophase. Although simultaneous localization of Cut12 and Pcp1 to SPBs was observed in both cases, Plo1 was not recruited (Figure 5B). Moreover, *pcp1-18* cells at the MI onset did not show a clear reduction of localization of Plo1 to SPBs, unlike mitotic *pcp1-18* cells (Fong *et al*., 2010; Figures 5C and S6, B and C).

We then tethered Plo1 to SPBs during meiotic prophase by constructing a fusion of Plo1 and Sid4 (Chang and Gould, 2000; Tomlin *et al*., 2002). The N-terminus of Sid4 is known to interact with Plo1 to recruit it to mitotic SPBs, whereas the C-terminus is the SPB-binding domain (Tomlin *et al*., 2002; Morrell *et al*., 2004). During meiotic prophase, Sid4 remaining partially on SPBs did not recruit Plo1. We therefore fused the C-terminal portion of Sid4 (Sid4C) and Plo1 to force Plo1 to localize to SPBs at meiotic prophase (Figure 5D). As expected, Sid4C-fused Plo1 (Plo1-Sid4C) localized to SPBs during meiotic prophase (Figure 5E). Interestingly, both Cut12 and Pcp1 localized to SPBs when Plo1-Sid4C was expressed (Figure 5E), suggesting that Plo1 recruited Cut12 and Pcp1 to SPBs, rather than vice versa. Cut12 and Pcp1 recruitment was not observed in control cells in which either Plo1 or Sid4C was expressed alone (Figure 5E). In addition, no effect was observed when inactive Plo1 was targeted to SPBs (Figure 5E). Hence the function of Plo1 for recruiting Cut12 and Pcp1 was exerted only when active Plo1 was localized to SPBs. This was further confirmed by using the *plo1.ts2* temperature-sensitive mutant (MacIver *et al*., 2003), in which Pcp1 displayed a significant delay in the reappearance to SPBs (Figure 5F). Notably, the extent of delay in relocalization of Pcp1 in *plo1.ts2* was similar to that in the *cdc31.ts10* mutant (Figure 4C), implying that the half-bridge–dependent localization of Plo1 to SPBs may induce reassociation of Pcp1 and Cut12 (Figure S6D).

Taken together, these results indicate that the localization dependency between Pcp1-Cut12 and Plo1 during meiosis may be distinct from that during mitosis: Pcp1 and Cut12 on SPBs recruit Plo1 upon entry into the mitotic cycle, whereas Plo1 on SPBs is required for relocalization of Pcp1 and Cut12 upon entry into meiosis. When Plo1 was artificially targeted to SPBs, not only Cut12 and Pcp1, but also Spo15, were recruited (Figure S6E). Thus we conclude that Plo1 may be a key factor for remodeling SPBs at the entry into MI.

Localization of Plo1 during prophase causes overamplification of SPBs

Using the engineered Plo1-targeting system, we then focused on the original question of why Plo1 is excluded from SPBs during meiotic prophase. When Plo1-Sid4C was expressed during meiosis, the zygotes frequently generated aberrant asci containing more than four nuclear masses (Figure 6A). This effect was not observed when the kinase-inactive form of Plo1 was fused to Sid4C (KD-Sid4C; Figure 6A). To determine the source of origination of this phenotype, we used live-cell imaging for the strains. Control cells expressing Sid4C-GFP (without Plo1) completed meiosis normally; Pcp1-CFP was dispersed throughout the cell during prophase (Figure 5E), then reappeared on SPBs prior to the onset of MI and was split into two dots when SPBs separated (Figure 6B). In contrast, when cells expressing Plo1-Sid4C-GFP entered MI, the Pcp1-CFP signal was abnormally split into multiple dots (in 58% of zygotes, n = 29; Figure 6B), indicating that SPBs were either fragmented or overduplicated. The number of amplified dots was either four (34%) or three (24%), but never more than four, suggesting that instead of undergoing random fragmentation, SPBs probably duplicated one more time than in normal cells. All of the four dots contained the half-bridge structure, as indicated by the Sfi1-CFP signal, similar to normal SPBs (Figure S7A). Prior to SPB separation, Pcp1-GFP localized at SPBs significantly increased both intensity (1.5-fold) and size (twofold), compared with the control cells expressing Sid4C alone (Figure S7B). Dysfunction of the half-bridge structure in *cdc31.ts10* cells at the restrictive temperature partially suppressed the generation of asci with missegregated chromosomes caused by Plo1-Sid4C-GFP

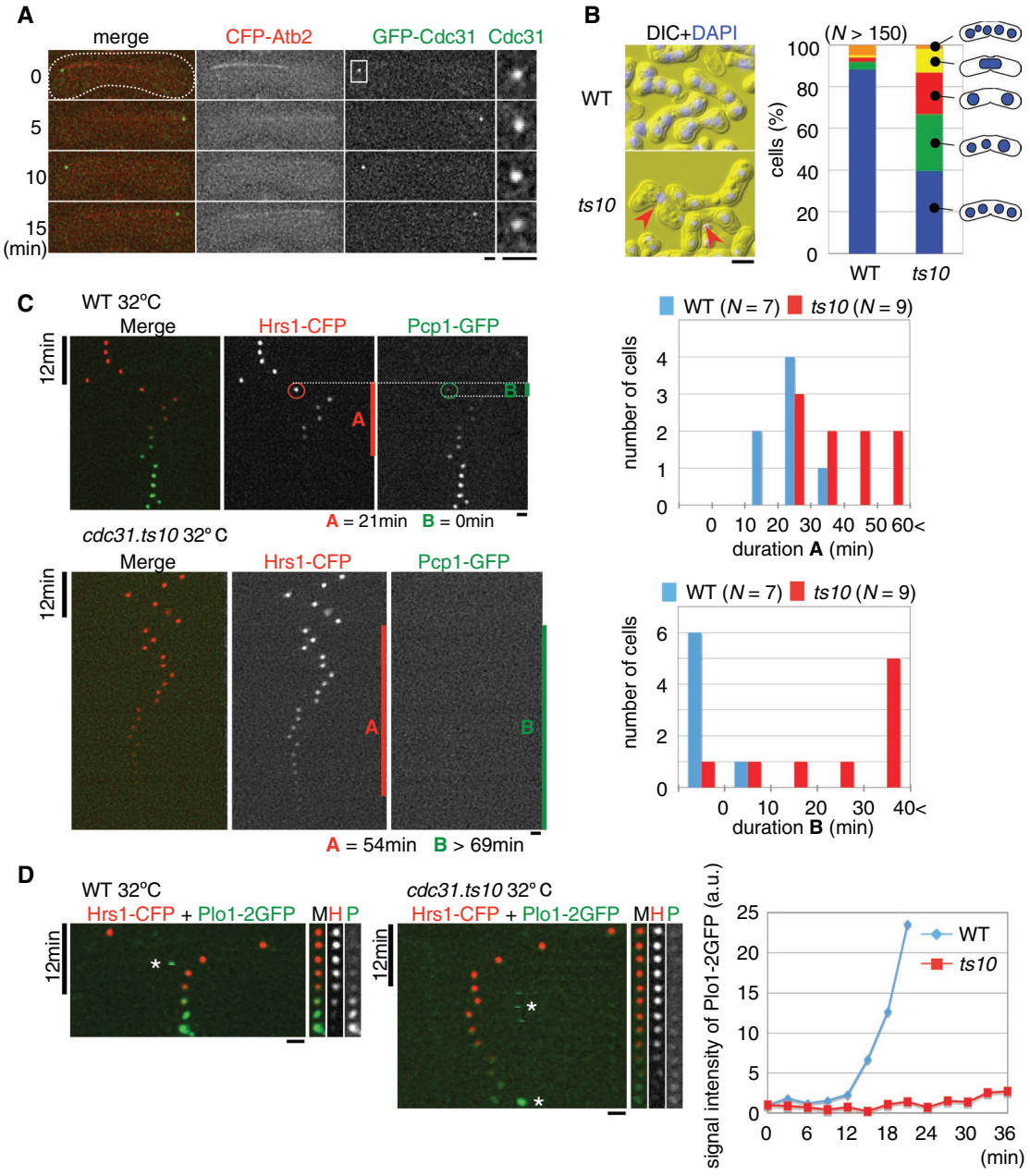

FIGURE 4: The SPB half-bridge component Cdc31 is required for reaccumulation of SPB components at the onset of MI. (A) GFP-Cdc31 along with CFP-Atb2 was filmed during meiotic prophase. The GFP-Cdc31 signals persisted at SPB during meiotic prophase (100% of cells, n = 17). (B) Meiotic phenotype of the *cdc31.ts10* mutant. Meiosis of wild-type and *cdc31.ts10* strains was induced. The cells were stained with DAPI, and the nuclei was counted. (C) Kymographs of Pcp1-GFP and Hrs1-CFP of the prophase/MI transition in wild-type and *cdc31.ts10* cells. The duration from the cessation of nuclear/SPB oscillation (red circle) to the disappearance of Hrs1-CFP localization at SPB is defined as duration A. The duration from the cessation of oscillation to the reappearance of Pcp1-GFP is defined as duration B. Cells were classified by durations A and B, and their distributions are shown in the graph. (D) Kymographs of Plo1-2GFP (P) and Hrs1-CFP (H) of the prophase/MI transition in wild-type and *cdc31.ts10* cells. Fifty-eight percent of *cdc31.ts10* cells showed a significant delay in localization of Plo1-2GFP to SPBs (n = 12). Asterisks denote Plo1-2GFP foci at kinetochores. To exclude the off-site signals of Plo1-2GFP, we selected the SPB region to align to the right. M, merged image. The fluorescence intensity of Plo1-2GFP specifically at SPBs was quantified. Scale bars: 1 μm (A, C, and D) or 5 μm (B).

(Figure 6, A and C). The viability of spores was also partially suppressed by introducing the *cdc31.ts10* mutation (*cdc31*$^{+}$ +Plo1-Sid4C-GFP: 19%, *cdc31.ts10* +Plo1-Sid4C-GFP: 31%). Furthermore, SPBs overamplified due to Plo1-Sid4C-GFP retained their ability to nucleate microtubules (Figure 6D) and therefore caused multipolar divisions during meiosis, which resulted in chromosome missegregation. Together these results suggest that SPBs underwent extra duplications before the onset of MI when Plo1 was constitutively localized to SPBs during meiotic prophase.

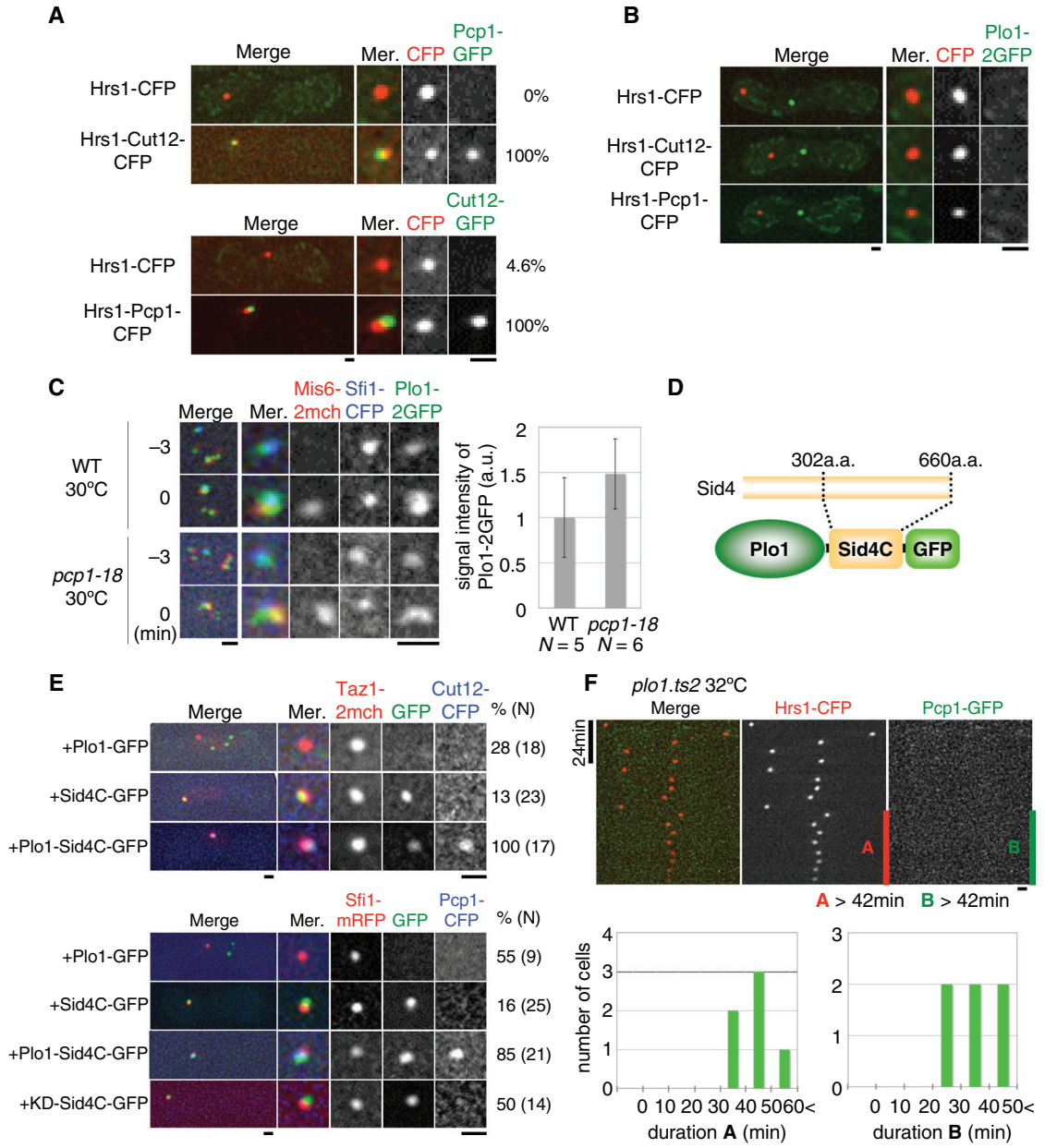

FIGURE 5: Plo1 at SPBs recruits Pcp1 and Cut12 to SPBs during meiosis. (A) Cut12 and Pcp1 were fused with Hrs1 to artificially localize them to prophase SPBs. Top, Hrs1-Cut12 (visualized by CFP) was monitored with Pcp1-GFP. Bottom, Hrs1-Pcp1-CFP was monitored with Cut12-GFP. Hrs1-CFP strains are shown as controls without the fusion. The regions corresponding to SPBs are magnified at the right. Percentages of cells with the Pcp1-GFP (top) or Cut12-GFP (bottom) dot are shown on the far right ($n \geq 31$). (B) Plo1-2GFP localization was observed when Hrs1-Cut12-CFP or Hrs1-Pcp1-CFP was expressed during meiotic prophase. Note that Plo1-2GFP was not recruited to SPBs (100% of cells, $n \geq 21$). (C) Plo1-2GFP fluorescence intensity during the prophase/MI transition in wild-type and the *pcp1-18* temperature-sensitive mutant was quantified. Cells were observed at 30°C. The timing of the onset of MI was defined as 0 min. The signal intensity of Plo1-2GFP at 3 min before the onset of MI (–3 min) was quantified and shown in the graph. No significant difference in Plo1-2GFP intensity was observed in wild-type and *pcp1-18* cells. Error bars = SD. (D) Schematic drawing for the Plo1-Sid4C fusion protein. a. a., amino acids. (E) The Plo1-Sid4C-GFP fusion protein was expressed with either Cut12-CFP or Pcp1-CFP. Taz1-2 mCherry and Sfi1-mRFP indicate the SPB position. Strains expressing Plo1-GFP and Sid4C-GFP are shown as controls. KD, the kinase-inactive mutant form of Plo1. Percentages of cells with the Cut12-CFP (top) or Pcp1-CFP (bottom) dot are shown on the right. (F) Kymographs of Pcp1-GFP and Hrs1-CFP during the prophase/MI transition of the *plo1.ts2* mutant. Durations A and B were defined and shown as in Figure 4C. Scale bars: 1 μm.

DISCUSSION

SPBs are remodeled in a meiosis-specific manner

This study illuminates two novel aspects of meiotic cell cycle regulation in fission yeast. First, we found that SPBs were remodeled in a meiosis-specific manner: some components dissociate from SPBs in meiotic prophase and reassociate to them upon MI entry. Second, we found that the polo kinase Plo1 and the centrin Cdc31 on the SPB half-bridge were required for the recovery process

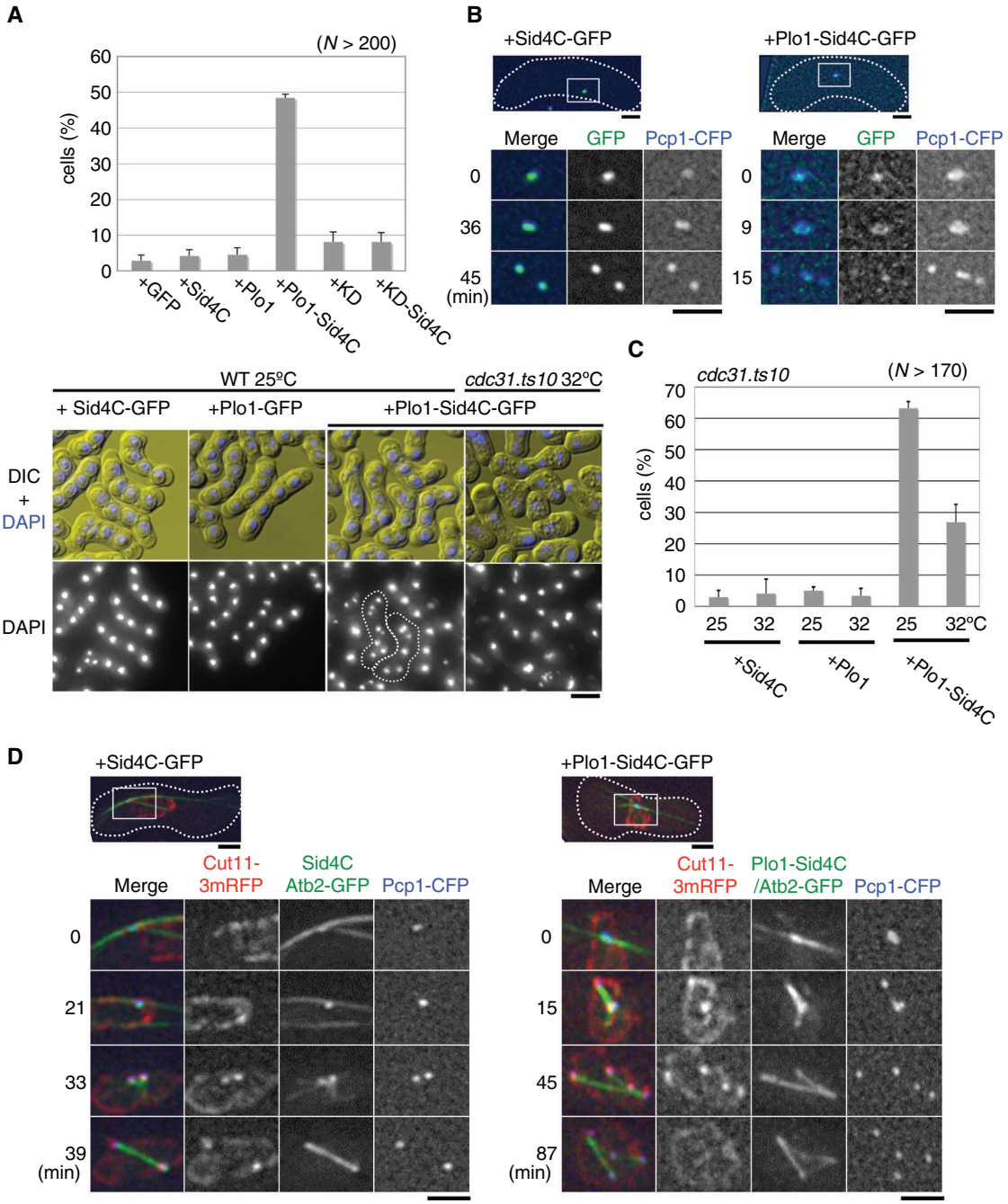

FIGURE 6: Ectopic localization of Plo1 at prophase SPB causes overamplification of SPB. (A) Meiosis was induced for cells expressing the indicated constructs. The cells were stained with DAPI, and the percentages of asci abnormally displaying more than four nuclei are shown in the graph. Differential interference contrast (DIC) and DAPI images of cells expressing the indicated constructs are shown at the bottom. DIC/DAPI images of *cdc31.ts10* cells expressing Plo1-Sid4C-GFP are also shown. (B) Pcp1-CFP was monitored at the onset of MI in cells expressing Plo1-Sid4C-GFP or Sid4C-GFP. Four foci of Pcp1-CFP were observed when Plo1-Sid4C-GFP was expressed. (C) Plo1-Sid4C and the control constructs were expressed in the *cdc31.ts10* mutant, and abnormal asci with more than five nuclei were counted as in (A). Error bars = SD. (D) Microtubules were visualized with GFP-Atb2 in cells expressing the fusion constructs (Sid4C-GFP for a control), Pcp1-CFP along with Cut11-3mRFP (a marker for the nuclear envelope and M-phase SPBs). +Plo1-Sid4C cells (67%) showed a multi-polar spindle, which was never seen in +Sid4C cells. Scale bars: 5 μm (A) or 2 μm (B and D).

of SPB remodeling, which revealed a novel function of these factors.

There are SPB components that constantly localize to SPBs, even though their functions appear to be limited to a part of the cell cycle. Our study has revealed that many factors in this category change their location during meiosis. Cut12, an activator of Plo1 (MacIver *et al.*, 2003), and Pcp1, a recruiter of Plo1 and γ-TuC (Fong *et al.*, 2010), function only during M-phase, although they localize constantly to SPBs throughout the mitotic cell cycle. Spo15 is required only during MII for SPB modification to enable assembly of forespore

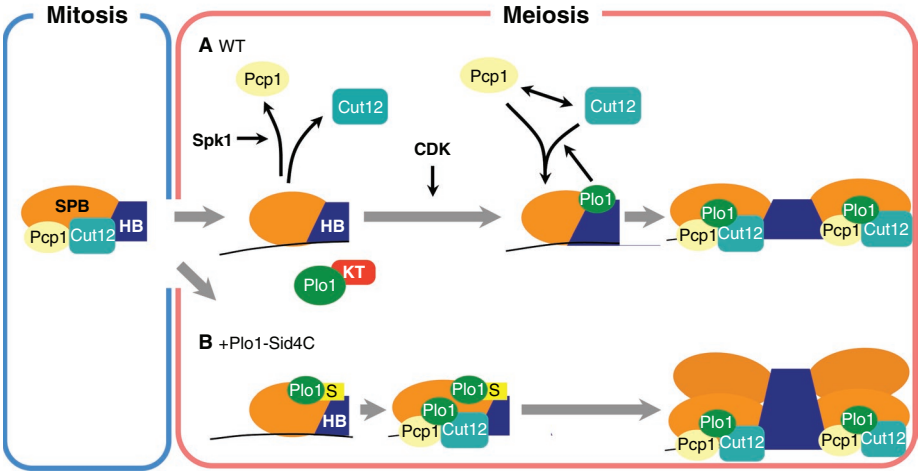

FIGURE 7: The model for the SPB reorganization in meiosis. Several SPB components, represented by Cut12 and Pcp1, constantly localize to SPBs (orange oval) during the mitotic cell cycle. (A) On meiotic commitment of wild-type (WT) cells, the MF-responsive MAP kinase Spk1 triggers the removal of SPB components, such as Pcp1 and Cut12, from the SPB. Polo kinase Plo1 localizes to kinetochores (KT) at this stage but is excluded from the SPB. At MI entry, the SPB components return to the SPB. The cyclin-dependent kinase (CDK) is required for the reconstruction of SPBs. CDK is known to activate Plo1 in fission yeast, and the reorganization process may start with the localization of Plo1 at the SPB, which requires Cdc31, a component of the SPB half-bridge (HB). Localization of Plo1 to SPB then induces the relocalization of Cut12 and Pcp1 (and other factors) to the SPB. Cut12 and Pcp1 mutually recruit each other to SPBs. The reconstruction then leads to duplication of the SPB. (B) When Plo1 is artificially targeted to the SPB by fusion of Sid4C (Plo1-Sid4C) in meiotic prophase, Cut12, Pcp1, and other components precociously localize to the SPB. This leads to the overamplification of SPBs by the onset of MI.

membrane and is dispensable for vegetative growth (Ikemoto *et al.*, 2000), yet it localizes to SPBs, even during the mitotic cycle. When cells are committed to sexual differentiation, Cut12, Pcp1, and Spo15 disappear from SPBs, remain absent during meiotic prophase, and return to SPBs immediately before the onset of MI. Sid4, a mitotically constitutive SPB component that functions for the SIN, also reduced its localization to SPBs in meiotic prophase. In contrast to these components, Hrs1 localizes to SPBs only during meiotic prophase (Saito *et al.*, 2005; Tanaka *et al.*, 2005). These observations indicate that SPB is remodeled in a meiosis-specific manner (Table 1).

Remodeling of the SPB components

Interestingly, similar to the SPB components analyzed in this study, a certain subset of kinetochore components is known to disperse from kinetochores during meiotic prophase, which is triggered by MF-responsive MAP kinase (Asakawa *et al.*, 2005; Hayashi *et al.*, 2006), although it remains unclear how Spk1 removes them. A number of kinetochore and SPB components have consensus sites for phosphorylation by MAP kinases. Thus it would be intriguing to test the possibility of whether Spk1 directly phosphorylates these components. Alternatively, Spk1 may alter the gene expression profile to adapt to changes in the environment, as is often seen with various MAP kinases. Genes regulated by Spk1 may affect the localizations of kinetochore and SPB components.

The recovery of these SPB components prior to MI requires the activity of the cyclin-dependent kinase Cdc2 and the intact half-bridge structure, which appears to constantly maintain its components during prophase. It has been shown that Cdc2 is required for activating Plo1 in fission yeast (Tanaka *et al.*, 2001), which supports Plo1 being a key factor for the reassociation of the SPB components upon entry into MI. Because it localizes to SPBs, Plo1 may directly phosphorylate SPB proteins. Cut12 activates Plo1, and Pcp1 recruits Plo1 to SPBs during mitosis (MacIver *et al.*, 2003; Fong *et al.*, 2010). During meiosis, however, Plo1 recruits these factors. We speculate that Plo1 induces reaccumulation of SPB components upon entry into MI and that when the SPB components, including Cut12 and Pcp1, return to the SPB, these Plo1 regulator proteins may further strengthen the effects of Plo1 via a positive-feedback mechanism in order to either induce maturation of SPBs or nucleate spindle microtubules.

Biological rationale for Plo1 exclusion from SPBs

In cells growing vegetatively, Plo1 localizes to SPBs during early mitosis (Bähler *et al.*, 1998a; Mulvihill *et al.*, 1999). During meiotic prophase, Plo1 localizes to the nucleoplasm and kinetochores (Krapp *et al.*, 2010) but is excluded from SPBs until the cell enters MI. The biological meaning and molecular mechanism of this Plo1 exclusion from SPBs has been enigmatic. As described above, artificial targeting of Plo1 to SPBs during meiotic prophase brought about an interesting interpretation regarding these questions. When Plo1 was forced to SPBs during prophase, it recruited Cut12, Pcp1, and other factors to SPBs. Thus Plo1 forced to SPBs precociously reorganized SPBs in preparation for upcoming MI, which ultimately resulted in an overduplication of SPBs. Although we could not completely exclude the possibility that the phenotype caused by the fusion protein might be due to off-target effects, the requirement of Plo1 kinase for the Pcp1 recruitment is supported by genetic evidence that Pcp1 did not relocalize to SPBs upon mitotic entry of the *plo1.ts2* mutant (Figure 5F) and that Plo1 localization did not depend on Pcp1 in meiosis (Figure 5C). Taking these results together, we propose that the relationship between Pcp1 and Plo1 may be distinct in mitosis and meiosis.

Thus Plo1 may play certain roles at kinetochores during meiotic prophase, while at the same time it needs to be excluded from SPBs until late prophase to ensure their proper duplication, the failure of which exerts a significant influence on chromosome segregation and production of descendants.

SPB remodeling and duplication

As illustrated in Figure 7, we propose that Plo1 induces reaccumulation of the SPB components at entry into MI. Because ectopic localization of Plo1 at SPBs induced both accumulation of the SPB components and SPB overduplication, these two events might be mechanically linked. It is noteworthy that the half-bridge component Cdc31/centrin is required for both SPB duplication and meiotic SPB remodeling. When an SPB is duplicated, a large number of the SPB components must accumulate to assemble a new SPB. This might be mechanistically similar to SPB remodeling at the onset of MI, in which a number of SPB components need to reaccumulate in order to reconstruct functional SPBs for upcoming MI.

An overexpression of the human or fly polo-like kinase Plk4/Sak causes an overduplication of centrioles (Kleylein-Sohn *et al.*, 2007; Peel *et al.*, 2007; Rodrigues-Martins *et al.*, 2007). Plk4 promotes

both canonical duplication and de novo biogenesis of centrioles. We envision that SPB remodeling prior to MI could be interpreted as "semi–de novo" biogenesis of SPB, because a number of components are absent or reduced, while the half-bridge components persist. Fission yeast Plo1 is not required for mitotic SPB duplication, but is specifically required for semi–de novo SPB biogenesis, in which SPBs are remodeled from the meiotic prophase, possibly by allowing reassociation of dispersed components.

It remains to be determined how Cdc31 is involved in this process. It is interesting to postulate that the half-bridge functions as a loading foothold for Plo1 at the SPB, at which cyclin-dependent kinase (CDK) and/or Plo1 might phosphorylate Cdc31 or other half-bridge components. However, recombinant GST (glutathione *S*-transferase)-Cdc31 was not successfully phosphorylated by these kinases in our in vitro kinase assay (unpublished data). Further investigation of meiotic SPB remodeling could find potential clues for how SPBs are duplicated during the cell cycle.

A question arises here of whether SPB remodeling during meiosis has other purposes. Dissociation of the SPB components could be essential for karyogamy, a process led by SPB and microtubules. Prophase cells expressing Plo1-Sid4C-GFP, however, displayed a single nucleus, indicating that karyogamy was not affected by ectopic localization of Plo1 to SPBs (Figure 6D). Another possible reason for prophase-specific SPB remodeling is for the nucleus to undergo SPB-driven horse-tail movement, which presumably promotes meiotic recombination of homologous chromosomes during prophase. Indeed, the prophase nucleus did not show frequent oscillatory movements when Plo1 was forced to localize to SPBs (unpublished data), which may have caused inefficient meiotic recombination. Thus, artificially tethered Plo1 results in multiple abnormalities, indicating that both the presence and absence of Plo1 regulate various aspects of meiosis. Hence we propose that Plo1, the localization of which is dependent on the stage of meiotic progression, may serve as the critical molecular switch that triggers SPB reorganization and enables its functioning.

MATERIALS AND METHODS

Yeast genetics, media, strains, and plasmids

The strains used in this study are listed in Supplemental Table S1. We used standard methods for yeast genetics and gene tagging (Moreno *et al*., 1991; Bähler *et al*., 1998b; Sato *et al*., 2005). The methods used for tagging tandem copies of fluorescent protein were described previously (Sato *et al*., 2009b). For visualization of GFP-tubulin, the gene for GFP-Atb2 (α2-tubulin), regulated by the *atb2*$^+$ promoter and terminator, was integrated into a chromosome as an extra copy of endogenous *atb2*$^+$ (referred to as "Z2-GFP-Atb2"). To induce mating, meiosis, and sporulation, we grew homothallic (*h*^{90}) strains in rich yeast extract medium supplemented with adenine (YEA) and spotted onto sporulation agar (SPA); this was followed by incubation at 25°C, 30°C, or 32°C.

For isolation of the temperature-sensitive *cdc31* mutant, a DNA fragment containing the *cdc31*$^+$ coding region, the *adh1* terminator and the marker gene *kan* was prepared by PCR and randomly mutagenized by an error-prone PCR amplification process (40 cycles) using Ex Taq DNA polymerase (Takara Bio). The wild-type strain JY741 was transformed with the amplified fragments. Approximately 10,000 transformants were screened for temperature sensitivity at 36°C. The isolated temperature-sensitive mutants were backcrossed with the wild-type strain JY878 to ensure proper integration and to remove potential off-site mutations. The viability of spores was tested as follows: *cdc31*$^+$ and *cdc31.ts10* cells expressing Plo1-Sid4C-GFP were spotted onto SPA and incubated at 32°C to induce mating and spore formation. Then 1×10^3 generated spores were plated onto YEA for each strain and incubated at 26.5°C. The number of colonies was counted to calculate the viability.

To visualize Cdc31 with GFP, we cloned *cdc31*$^+$ cDNA into the plasmid pKNKN, by which was used to express the GFP-Cdc31 fusion gene from the *nmt1* promoter. The plasmid was then linearized with *Eco*RV and integrated into the *lys1* locus of chromosome 1. The cells were grown in YEA, spotted onto SPA, and observed in minimal medium EMM.

The fusion protein constructs Hrs1-Cut12 and Hrs1-Pcp1 (Figure 5, A and B) were created by amplifying the coding region for Cdc12 and Pcp1 and then cloning it into the PacI restriction site of the plasmid pFA6a-CFP-*nat* (Sato *et al*., 2005). Using the resultant plasmids (pFA6a-Cut12-CFP-*nat* and pFA6a-Pcp1-CFP-*nat*) and standard gene-targeting procedures (Bähler *et al*., 1998b), we tagged the endogenous *hrs1*$^+$ gene with Cut12-CFP and Pcp1-CFP genes with *nat* as the selection marker to create the in-frame fusion genes of Hrs1-Cut12-CFP and Hrs1-Pcp1-CFP, respectively. Figure 5, A and B, shows experiments performed in the *mei4*Δ background in order to arrest cells prior to their entry into MI. To create the fusion construct of Plo1-Sid4C, we used the C-terminal part (302–660 amino acids), known to be the SPB-docking domain for Sid4 (Tomlin *et al*., 2002). Sid4C was cloned into the plasmid pREP41-GFP containing a DSR (determinant of selective removal) element, which is a part of *mei4*$^+$, eliminated by the Mmi1-exosome system (Harigaya *et al*., 2006). Next the coding region of Plo1 was cloned into the above plasmid to create pREP41-Plo1-Sid4C-GFP-DSR.

For Plo1 overexpression, the coding region of Plo1 fused with a DSR element was cloned into the expression vector pREP41. Transformants carrying this construct were grown in EMM supplemented with a nitrogen source, spotted onto SPA plates, and incubated for 5–6 h prior to observation.

Microscopy

For visualization of the cellular DNA of meiotic cells by fluorescence microscopy, yeast cells were spotted onto SPA. After 24 h, the cells were fixed with ice-cold ethanol and stained with 4′,6-diamidino-2-phenylindole (DAPI; Wako Pure Chemicals). An Axioplan 2 fluorescence microscope (Zeiss) and SlideBook software (Leeds Precision) were used to acquire images. Live-cell imaging used the DeltaVision-SoftWoRx system (Applied Precision), as described previously (Sato *et al*., 2009b). Images were acquired as serial sections along the *z*-axis and stacked using the Quick projection algorithm in SoftWoRx. The captured images were processed with Adobe Photoshop CS2 (version 9.0; San Jose, CA).

For observation of mitotic cells (Figures 2A, S2, and S6B), cells were grown in synthetic medium supplemented with adenine, leucine, and uracil at 25°C, and were observed in the same medium at 25°C or 36°C, as indicated. For observation of meiotic cells, cells were spotted onto SPA. After 9–11 h, cells were observed by microscopy. EMM with supplements was used during observation. For the experiments illustrated in Figures 3A and S3 examining the involvement of MAP kinase Spk1, the strains were grown in liquid YEA at 25°C. For +N samples, cells were observed with EMM+N. The –N samples were washed five times with EMM+ALU without the nitrogen source ammonium and then inoculated in EMM+ALU without (–N) ammonium chloride. After 7 h of incubation, cells were observed using the same media. For Figure 3B, *cdc2-as* strain MO1885 was spotted onto SPA at 25°C. Cells were mounted on a glass-bottom dish and observations were initiated by focusing on cells entering MI, as previously described (Sato *et al*., 2009a). Next dimethyl sulfoxide was added to a mock-treated sample (–analogue) or 2 μM

1NM-PP1 (Calbiochem) was added to inhibit the activity of Cdc2 kinase (+analogue). For experiments using strains with plasmids (Figures 1B, 5E, 6, A–D, S4, S6E, and S7), cells were grown in liquid EMM (+N) supplemented with adenine and uracil (EMM+N+AU) for 12–14 h to induce the expression under the *nmt1* promoter or derivatives. Cells were then washed twice with water and spotted onto SPA at 25°C or 32°C. After 5–8 h, cells were observed by microscopy. The EMM+AU without (–N) ammonium chloride was used for these observations.

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REFERENCES

Adams IR, Kilmartin JV (2000). Spindle pole body duplication: a model for centrosome duplication? Trends Cell Biol 10, 329–335.

Araki Y, Gombos L, Migueleti SP, Sivashanmugam L, Antony C, Schiebel E (2010). N-terminal regions of Mps1 kinase determine functional bifurcation. J Cell Biol 189, 41–56.

Asakawa H, Hayashi A, Haraguchi T, Hiraoka Y (2005). Dissociation of the Nuf2-Ndc80 complex releases centromeres from the spindle-pole body during meiotic prophase in fission yeast. Mol Biol Cell 16, 2325–2338.

Bähler J, Steever AB, Wheatley S, Wang Y, Pringle JR, Gould KL, McCollum D (1998a). Role of polo kinase and Mid1p in determining the site of cell division in fission yeast. J Cell Biol 143, 1603–1616.

Bähler J, Wu JQ, Longtine MS, Shah NG, McKenzie A, III, Steever AB, Wach A, Philippsen P, Pringle JR (1998b). Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. Yeast 14, 943–951.

Bardin AJ, Amon A (2001). MEN and SIN: what's the difference? Nat Rev Mol Cell Biol 2, 815–826.

Bishop AC *et al.* (2000). A chemical switch for inhibitor-sensitive alleles of any protein kinase. Nature 407, 395–401.

Bornens M (2012). The centrosome in cells and organisms. Science 335, 422–426.

Cerutti L, Simanis V (1999). Asymmetry of the spindle pole bodies and spg1p GAP segregation during mitosis in fission yeast. J Cell Sci 112, 2313–2321.

Chang L, Gould KL (2000). Sid4p is required to localize components of the septation initiation pathway to the spindle pole body in fission yeast. Proc Natl Acad Sci USA 97, 5249–5254.

Chikashige Y, Ding DQ, Funabiki H, Haraguchi T, Mashiko S, Yanagida M, Hiraoka Y (1994). Telomere-led premeiotic chromosome movement in fission yeast. Science 264, 270–273.

Compton DA (2000). Spindle assembly in animal cells. Annu Rev Biochem 69, 95–114.

Decottignies A, Zarzov P, Nurse P (2001). In vivo localisation of fission yeast cyclin-dependent kinase cdc2p and cyclin B cdc13p during mitosis and meiosis. J Cell Sci 114, 2627–2640.

Ding DQ, Chikashige Y, Haraguchi T, Hiraoka Y (1998). Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells. J Cell Sci 111, 701–712.

Ding R, West RR, Morphew DM, Oakley BR, McIntosh JR (1997). The spindle pole body of *Schizosaccharomyces pombe* enters and leaves the nuclear envelope as the cell cycle proceeds. Mol Biol Cell 8, 1461–1479.

Dischinger S, Krapp A, Xie L, Paulson JR, Simanis V (2008). Chemical genetic analysis of the regulatory role of Cdc2p in the *S. pombe* septation initiation network. J Cell Sci 121, 843–853.

Fletcher L, Muschel RJ (2006). The centrosome and the DNA damage induced checkpoint. Cancer Lett 243, 1–8.

Flory MR, Morphew M, Joseph JD, Means AR, Davis TN (2002). Pcp1p, an Spc110p-related calmodulin target at the centrosome of the fission yeast *Schizosaccharomyces pombe*. Cell Growth Differ 13, 47–58.

Fong CS, Sato M, Toda T (2010). Fission yeast Pcp1 links polo kinase-mediated mitotic entry to γ-tubulin-dependent spindle formation. EMBO J 29, 120–130.

González C, Tavosanis G, Mollinari C (1998). Centrosomes and microtubule organisation during *Drosophila* development. J Cell Sci 111, 2697–2706.

Guertin DA, Chang L, Irshad F, Gould KL, McCollum D (2000). The role of the sid1p kinase and cdc14p in regulating the onset of cytokinesis in fission yeast. EMBO J 19, 1803–1815.

Harigaya Y, Tanaka H, Yamanaka S, Tanaka K, Watanabe Y, Tsutsumi C, Chikashige Y, Hiraoka Y, Yamashita A, Yamamoto M (2006). Selective elimination of messenger RNA prevents an incidence of untimely meiosis. Nature 442, 45–50.

Hayashi A, Asakawa H, Haraguchi T, Hiraoka Y (2006). Reconstruction of the kinetochore during meiosis in fission yeast *Schizosaccharomyces pombe*. Mol Biol Cell 17, 5173–5184.

Heuer JG, Li K, Kaufman TC (1995). The *Drosophila* homeotic target gene *centrosomin* (*cnn*) encodes a novel centrosomal protein with leucine zippers and maps to a genomic region required for midgut morphogenesis. Development 121, 3861–3876.

Hirata D *et al.* (2002). Fission yeast Mor2/Cps12, a protein similar to *Drosophila* Furry, is essential for cell morphogenesis and its mutation induces Wee1-dependent G_2 delay. EMBO J 21, 4863–4874.

Ikemoto S, Nakamura T, Kubo M, Shimoda C (2000). *S. pombe* sporulation-specific coiled-coil protein Spo15p is localized to the spindle pole body and essential for its modification. J Cell Sci 113, 545–554.

Jaspersen SL, Martin AE, Glazko G, Giddings TH, Jr., Morgan G, Mushegian A, Winey M (2006). The Sad1-UNC-84 homology domain in Mps3 interacts with Mps2 to connect the spindle pole body with the nuclear envelope. J Cell Biol 174, 665–675.

Jaspersen SL, Winey M (2004). The budding yeast spindle pole body: structure, duplication, and function. Annu Rev Cell Dev Biol 20, 1–28.

Kanai M, Kume K, Miyahara K, Sakai K, Nakamura K, Leonhard K, Wiley DJ, Verde F, Toda T, Hirata D (2005). Fission yeast MO25 protein is localized at SPB and septum and is essential for cell morphogenesis. EMBO J 24, 3012–3025.

Kilmartin JV (2003). Sfi1p has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. J Cell Biol 162, 1211–1221.

Kilmartin JV, Goh PY (1996). Spc110p: assembly properties and role in the connection of nuclear microtubules to the yeast spindle pole body. EMBO J 15, 4592–4602.

Kleylein-Sohn J, Westendorf J, Le Clech M, Habedanck R, Stierhof YD, Nigg EA (2007). Plk4-induced centriole biogenesis in human cells. Dev Cell 13, 190–202.

Kollman JM, Merdes A, Mourey L, Agard DA (2011). Microtubule nucleation by γ-tubulin complexes. Nat Rev Mol Cell Biol 12, 709–721.

Krapp A, Collin P, Cokoja A, Dischinger S, Cano E, Simanis V (2006). The *Schizosaccharomyces pombe* septation initiation network (SIN) is required for spore formation in meiosis. J Cell Sci 119, 2882–2891.

Krapp A, Del Rosario EC, Simanis V (2010). The role of *Schizosaccharomyces pombe dma1* in spore formation during meiosis. J Cell Sci 123, 3284–3293.

Krapp A, Schmidt S, Cano E, Simanis V (2001). *S. pombe* cdc11p, together with sid4p, provides an anchor for septation initiation network proteins on the spindle pole body. Curr Biol 11, 1559–1568.

Krapp A, Simanis V (2008). An overview of the fission yeast septation initiation network (SIN). Biochem Soc Trans 36, 411–415.

Leonhard K, Nurse P (2005). Ste20/GCK kinase Nak1/Orb3 polarizes the actin cytoskeleton in fission yeast during the cell cycle. J Cell Sci 118, 1033–1044.

MacIver FH, Tanaka K, Robertson AM, Hagan IM (2003). Physical and functional interactions between polo kinase and the spindle pole component Cut12 regulate mitotic commitment in *S. pombe*. Genes Dev 17, 1507–1523.

McCollum D, Gould KL (2001). Timing is everything: regulation of mitotic exit and cytokinesis by the MEN and SIN. Trends Cell Biol 11, 89–95.
Mendoza M, Redemann S, Brunner D (2005). The fission yeast MO25 protein functions in polar growth and cell separation. Eur J Cell Biol 84, 915–926.
Moreno S, Klar A, Nurse P (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol 194, 795–823.
Morrell JL *et al*. (2004). Sid4p-Cdc11p assembles the septation initiation network and its regulators at the *S. pombe* SPB. Curr Biol 14, 579–584.
Mulvihill DP, Petersen J, Ohkura H, Glover DM, Hagan IM (1999). Plo1 kinase recruitment to the spindle pole body and its role in cell division in *Schizosaccharomyces pombe*. Mol Biol Cell 10, 2771–2785.
Nurse P (1990). Universal control mechanism regulating onset of M-phase. Nature 344, 503–508.
Paoletti A, Bordes N, Haddad R, Schwartz CL, Chang F, Bornens M (2003). Fission yeast cdc31p is a component of the half-bridge and controls SPB duplication. Mol Biol Cell 14, 2793–2808.
Peel N, Stevens NR, Basto R, Raff JW (2007). Overexpressing centriole-replication proteins in vivo induces centriole overduplication and *de novo* formation. Curr Biol 17, 834–843.
Rodrigues-Martins A, Riparbelli M, Callaini G, Glover DM, Bettencourt-Dias M (2007). Revisiting the role of the mother centriole in centriole biogenesis. Science 316, 1046–1050.
Rose MD (1991). Nuclear fusion in yeast. Annu Rev Microbiol 45, 539–567.
Rosenberg JA, Tomlin GC, McDonald WH, Snydsman BE, Muller EG, Yates JR, III, Gould KL (2006). Ppc89 links multiple proteins, including the septation initiation network, to the core of the fission yeast spindle-pole body. Mol Biol Cell 17, 3793–3805.
Saito TT, Tougan T, Okuzaki D, Kasama T, Nojima H (2005). Mcp6, a meiosis-specific coiled-coil protein of *Schizosaccharomyces pombe*, localizes to the spindle pole body and is required for horsetail movement and recombination. J Cell Sci 118, 447–459.
Sato M, Dhut S, Toda T (2005). New drug-resistant cassettes for gene disruption and epitope tagging in *Schizosaccharomyces pombe*. Yeast 22, 583–591.
Sato M, Okada N, Kakui Y, Yamamoto M, Yoshida M, Toda T (2009a). Nucleocytoplasmic transport of Alp7/TACC organizes spatiotemporal microtubule formation in fission yeast. EMBO Rep 10, 1161–1167.
Sato M, Toya M, Toda T (2009b). Visualization of fluorescence-tagged proteins in fission yeast: the analysis of mitotic spindle dynamics using GFP-tubulin under the native promoter. Methods Mol Biol 545, 185–203.
Schatten G (1994). The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. Dev Biol 165, 299–335.
Snyder M (1994). The spindle pole body of yeast. Chromosoma 103, 369–380.
Sohrmann M, Schmidt S, Hagan I, Simanis V (1998). Asymmetric segregation on spindle poles of the *Schizosaccharomyces pombe* septum-inducing protein kinase Cdc7p. Genes Dev 12, 84–94.
Sparks CA, Morphew M, McCollum D (1999). Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. J Cell Biol 146, 777–790.
Takaki T, Trenz K, Costanzo V, Petronczki M (2008). Polo-like kinase 1 reaches beyond mitosis–cytokinesis, DNA damage response, and development. Curr Opin Cell Biol 20, 650–660.
Tamaskovic R, Bichsel SJ, Hemmings BA (2003). NDR family of AGC kinases–essential regulators of the cell cycle and morphogenesis. FEBS Lett 546, 73–80.
Tanaka K, Kohda T, Yamashita A, Nonaka N, Yamamoto M (2005). Hrs1p/Mcp6p on the meiotic SPB organizes astral microtubule arrays for oscillatory nuclear movement. Curr Biol 15, 1479–1486.
Tanaka K, Petersen J, MacIver F, Mulvihill DP, Glover DM, Hagan IM (2001). The role of Plo1 kinase in mitotic commitment and septation in *Schizosaccharomyces pombe*. EMBO J 20, 1259–1270.
Tomlin GC, Morrell JL, Gould KL (2002). The spindle pole body protein Cdc11p links Sid4p to the fission yeast septation initiation network. Mol Biol Cell 13, 1203–1214.
Toya M, Sato M, Haselmann U, Asakawa K, Brunner D, Antony C, Toda T (2007). γ-tubulin complex-mediated anchoring of spindle microtubules to spindle-pole bodies requires Msd1 in fission yeast. Nat Cell Biol 9, 646–653.
Verde F, Wiley DJ, Nurse P (1998). Fission yeast orb6, a ser/thr protein kinase related to mammalian rho kinase and myotonic dystrophy kinase, is required for maintenance of cell polarity and coordinates cell morphogenesis with the cell cycle. Proc Natl Acad Sci USA 95, 7526–7531.
West RR, Vaisberg EV, Ding R, Nurse P, McIntosh JR (1998). *cut11*$^{+}$: a gene required for cell cycle-dependent spindle pole body anchoring in the nuclear envelope and bipolar spindle formation in *Schizosaccharomyces pombe*. Mol Biol Cell 9, 2839–2855.
Wiese C, Zheng Y (2006). Microtubule nucleation: γ-tubulin and beyond. J Cell Sci 119, 4143–4153.
Yamamoto A, West RR, McIntosh JR, Hiraoka Y (1999). A cytoplasmic dynein heavy chain is required for oscillatory nuclear movement of meiotic prophase and efficient meiotic recombination in fission yeast. J Cell Biol 145, 1233–1249.
Yamamoto M, Imai Y, Watanabe Y (1997). Mating and sporulation in *Schizosaccharomyces pombe*. In: The Molecular and Cellular Biology of the Yeast *Saccharomyces*, Vol. 3: Cell Cycle and Biology, ed. JR Pringle, JR Broach, and EW Jones, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1037–1106.
Yamamoto TG, Chikashige Y, Ozoe F, Kawamukai M, Hiraoka Y (2004). Activation of the pheromone-responsive MAP kinase drives haploid cells to undergo ectopic meiosis with normal telomere clustering and sister chromatid segregation in fission yeast. J Cell Sci 117, 3875–3886.